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Detection of Adenoviruses and Rotaviruses in Drinking Water Sources Used In Rural Areas of Benin, West Africa▽

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Diseases associated with viruses also found in environmental samples cause major health problems in developing countries. Little is known about the frequency and pattern of viral contamination of drinking water sources in these resource-poor settings. We established a method to analyze 10 liters of water from drinking water sources in a rural area of Benin for the presence of adenoviruses and rotaviruses. Overall, 541 samples from 287 drinking water sources were tested. A total of 12.9% of the sources were positive for adenoviruses and 2.1% of the sources were positive for rotaviruses at least once. Due to the temporary nature of viral contamination in drinking water sources, the probability of virus detection increased with the number of samples taken at one test site over time. No seasonal pattern for viral contaminations was found after samples obtained during the dry and wet seasons were compared. Overall, 3 of 15 surface water samples (20%) and 35 of 247 wells (14.2%) but also 2 of 25 pumps (8%) tested positive for adenoviruses or rotaviruses. The presence of latrines within a radius of 50 m in the vicinity of pumps or wells was identified as being a risk factor for virus detection. In summary, viral contamination was correlated with the presence of latrines in the vicinity of drinking water sources, indicating the importance of appropriate decision support systems in these socioeconomic prospering regions.

Although access to safe drinking water is considered a human right, many people suffer from inadequate water supply. Especially in developing countries, improper water quality causes major public health problems affecting mortality rates in highly susceptible people (small children and immunocompromised patients) as well as economic income problems due to disease-related nonproductive time. Only 37.4% of households have access to piped water sources in Benin, West Africa, and in rural areas, even fewer have access (3). Many diseases like diarrhea, gastroenteritis, keratoconjunctivitis, respiratory infections, and hepatitis are associated with viruses, often found in environmental samples like groundwater, surface water, sewage, costal water, shellfish, and tap water (5, 6, 10, 13, 23, 38). Virus concentrations in environmental samples are low both due to the inability to replicate without a host cell and because of continuous degradation and dilution effects. On the other hand and in contrast to most bacterial infections, even small amounts of viruses (as few as 10 PCR-detectable units) are sufficient to establish an infection in the new host (24). Bacterial indicators seem to be inappropriate for analyzing viral contamination, since viruses are more resistant to environmental conditions (2) and spread over a longer distance than bacteria (9). Therefore, viruses are often found without any bacterial indicator for fecal contamination (2, 6). In North America, 15 to 30% of all gastrointestinal diseases were suspected of being related to water (30), whereas worldwide, over 88% of diarrheal diseases are waterborne or water related (18).

Routine screening of environmental samples for viral contamination is controversially being discussed at the moment. Furthermore, no standard procedure for the detection of viruses in environmental samples currently exists. In numerous studies, virus concentration from water was achieved by filtration using electropositive filters (1MDS) (12, 15, 25, 26, 33). Further methods using ultrafiltration, glass wool filters (7, 22), or immunomagnetic separation (16, 28) were used to detect small amounts of viruses independently of matrix effects. However, the U.S. Environmental Protection Agency listed adenovirus as one of nine microorganisms on the contamination candidate list for drinking water as a potential indicator virus due to an outstanding resistance to UV disinfection. The 51 presently recognized adenovirus serotypes are responsible for a great variety of human diseases like diarrhea, keratoconjunctivitis, and respiratory infections. However, severe diarrhea, especially in small children and immunocompromised patients, is often caused by rotaviruses. The fatal outcome of infant diarrhea substantially contributes to the high mortality rate of children under the age of 5 years in developing countries (8). In Benin, the probability of dying per 1,000 live births under 5 years of age (under-5 mortality rate) was 152 in 2004, and 17.1% of these deaths were caused by diarrheal diseases (37). To address the frequency and pattern of viral contamination in drinking water sources in rural areas of West Africa, we analyzed water samples during the dry and wet seasons in Benin for contamination with adenoviruses as

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indicators and rotaviruses as important pathogens in developing countries.

MATERIALS AND METHODS

Water concentration. Water samples ($n = 541$) were taken throughout the years 2003 to 2007 from 287 georeferenced drinking water sources (247 water wells, 25 pumps, and 15 surface water samples) located in 55 different villages between the cities of Parakou, Dogue, and Djogou of Benin, West Africa. Multiple samples were taken from 115 drinking water sources (median of three samples at each of these test sites). During the dry seasons, 225 samples were taken, and during the wet seasons, 316 samples were taken. Latrines located in the area analyzed were also georeferenced ($n = 220$). Ten liters of water each was collected, transported to the Impetus Laboratory in Parakou and passed through two 1MDS cartridge filters (Cuno filter system, ZetaPlusRVirosorbR; 3M, Germany) with a flow rate of 5 liters/h. The charged filters were eluted with 20 ml extraction buffer (1% powdered milk suspension; pH 9.5). After incubation for 5 min, the fluid was forced from the filter housing into a sterile tube by using compressed air.

RNA/DNA extraction and virus detection. RNA and DNA were extracted from 1 ml eluate with the commercially available MagNA Pure Total nucleic acid extraction kit (Roche Diagnostics) according to the manufacturer's instructions and eluted in 50 μl. Detection of adenovirus and rotavirus was performed as previously described (14, 29). For adenovirus detection, a PCR mix of 20 μl with 0.5 μl TaqMan probe (16 μM) (5′–6-carboxyfluorescein–TGCACCAGACCCG GGCTCAGGTACTCCGA–6-carboxytetramethylrhodamine–3′), 0.1 μl of each primer (100 μM) (AQ1 [5′-GCCACGGTGGGGTTTCTAAACTT-3′] and AQ2 [5′-GCCCCAGTGGTCTTACATGCACATC-3′]), 1.6 μl $MgCl_2$, 3.7 μl H_2O, and 2 μl enzyme mix (LightCycler FastStart DNA master kit; Roche) was used. The PCR for rotavirus detection was performed in a 20-μl volume containing 0.3 μl TaqMan probe (10 μM) (5′–6-carboxyfluorescein–ATGAGCACAATAGTT AAAAGCTAACACTGTCAA–6-carboxytetramethylrhodamine–3′), 0.4 μl of each primer (10 μM) (NVP3-F [5′-ACCATCTACACATGACCCTC-3′] and NVP3-R [5′-GGTCACATAACGCCCC-3′]), 1.3 μl $MnCl_2$, 5.1 μl H_2O, and 7.5 μl enzyme mix (LightCycler RNA master kit; Roche). The real-time PCR was carried out using a LightCycler apparatus (Roche) according to the above-described protocols. Due to the small amounts of viral nucleic acid, PCR was evaluated only for the presence or absence of viruses.

Statistical analysis. Statistical significance was determined using Fisher's exact test to analyze the influence of latrines within the vicinity of drinking water sources. Significance was considered at the 95% confidence level (P values of <0.05).

RESULTS

Drinking water sources in rural areas of Benin, West Africa, were analyzed for viral contamination. When tested for the first time, adenoviral DNA was detected in 26 out of 287 water sources (9.1%), and rotaviral RNA was detected in one water source. Twenty initially virus-positive water sources were resampled one to seven times. Only 3 of 47 water samples were positive. They came from three different sources sampled three to seven times and were taken 19, 21, and 22 months after the first positive sample. In one case, adenoviruses were found a second time, whereas rotaviruses were detected in two sources after the initial finding of adenoviruses.

We also resampled 95 drinking water sources, which tested negative at the beginning, one to eight times. Thirteen of these drinking water sources (13.7%) tested positive once during the observation period (10 with adenoviruses, 1 with adenoviruses and rotaviruses, and 2 with rotaviruses). The other 82 water sources and 194 samples tested negative for viral contamination.

Overall, 40 of 287 drinking water sources (13.9%) were positive for viral contamination at least once. Mainly adenoviruses were detected in drinking water sources (12.9%), whereas only a few tested positive for rotaviruses (2.1%) (Table 1).

TABLE 1. Drinking water sources with and without viral contamination

Source	No. of drinking water sources with contamination by:			No. of drinking water sources without viral contamination ($n = 247$)
	Adenoviruses ($n = 37$)	Rotaviruses ($n = 6$)	Viruses at least once ($n = 40$)	
Surface water	3	0	3	12
Pump	2	0	2	23
Well	32	6	35	212

Viral contaminations were found in all kinds of drinking water sources. The frequency of viral contamination did not significantly differ between water wells (14.2%; $n = 35$), surface water (20%; $n = 3$), and pumps (8.0%; $n = 2$) (Table 1). However, pumps seemed to be subject to viral contamination less often than other drinking water sources.

The analysis of viral detection according to the month of sample collection revealed no seasonal influence on the risk of viral contamination. A total of 20 of 225 samples (8.9%) taken from November to April during the dry season tested positive for viral contamination, compared to 23 of 316 samples (7.2%) collected from May to October during the wet season. Notably, two of three positive surface water samples were taken in the middle of the dry season (December and January).

Global positioning system (GPS) data for the drinking water sources (wells and pumps) ($n = 272$) were combined with GPS data for 220 latrines identified in the area under investigation. Latrines were either the private property of inhabitants or used by the community. According to GPS data, each drinking water source (pump or well) was assigned to the five nearest latrines (distance in meters). Georeferenced latrines and drinking water sources are shown for one of the 55 villages (Fig. 1). At least one latrine within a radius of 50 m of the water source was significantly more often found near wells or pumps that tested positive for viral contamination (12/37 versus 40/235; $P < 0.05$). To avoid any bias from the separated testing of a subset of water sources, the correlation between nearby latrines and virus contamination was confirmed on the basis of a single analysis of each water source (10/24 versus 42/248; $P < 0.01$). Notably, one of the three repeatedly positive drinking water sources was located 29 m from a latrine, and the second was 59 m from a latrine.

DISCUSSION

We found a considerable number of drinking water sources that were positive for adenoviruses or rotaviruses, of which adenoviruses clearly dominated. Since a "gold standard" for the detection of viruses in environmental samples is not yet defined, the prevalence of viral contamination might be underestimated by our methods (15, 16, 26). Furthermore, it was shown that the number of samples taken at one site influenced the probability of virus detection, also suggesting more drinking water sources in danger of viral contamination. Whereas our attempts to sequence positive samples failed due to low viral concentrations, other groups found all kinds of adenovi-

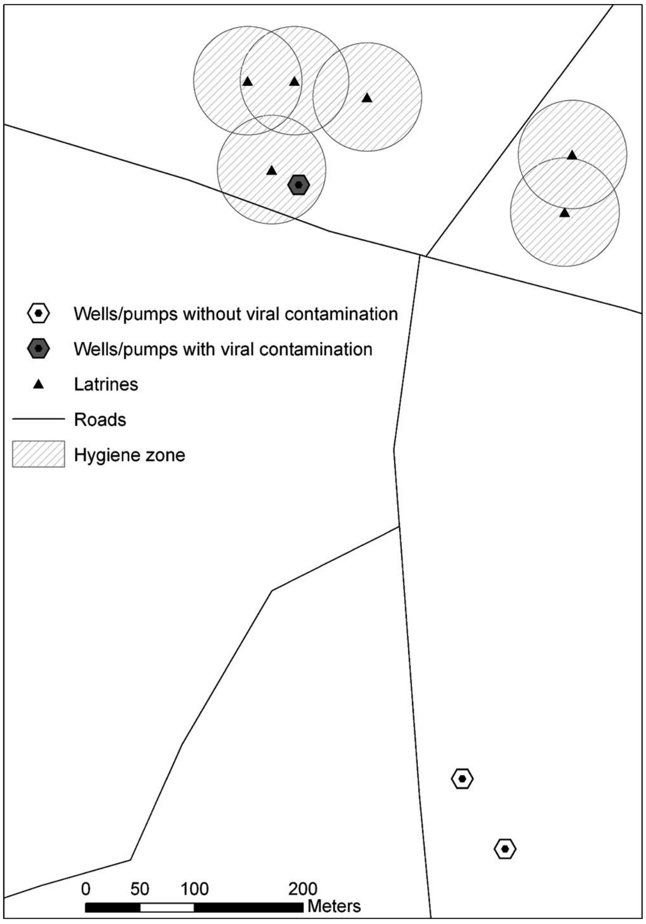

FIG. 1. Example of georeferenced drinking water sources (wells/pumps are indicated with hexagons with a dot) and latrines (triangles) surrounded by a 50-m hygiene zone in one of the 55 villages analyzed.

rus serotypes in environmental samples and not only adenovirus serotypes 40 and 41, which typically cause gastroenteritis (18, 34). This fact could explain the dominant detection of adenoviruses in our samples, since not only children with diarrhea but also adults with different kinds of adenovirus infections spread the virus to the environment, supporting the potential indicator character of adenoviruses.

Pathogens can reach drinking water sources by different pathways. Poor design or construction can lead to a rapid bypass mechanism further boosted by water flows in the soil and is called the localized pathway. On the other hand, migration of viruses through the subsoil to the water table can result in contamination of drinking water sources, named the aquifer pathway (11). Since we did not find any differences in viral contamination during the dry or wet season, both factors seem to be responsible for viral contamination in rural areas of Benin. Although during the wet season, water flow in the soil eased virus transport, the dilution effect hampered detection as well as infection (20, 32). In contrast, during the dry season, less water flow occurs, but contamination of drinking water sources by aquifer pathways is still possible, and fewer dilution effects hamper virus detection. It can be hypothesized that during the wet season, viruses are transported by water flows in the upper part of the soil, whereas during the dry season, contamination through the ground flow is more likely supported by the positive surface waters at this time of the year.

The 20% of adenovirus contamination in surface water found in this study was similar to previously described results (17, 19, 35, 36, 38). The absence of rotaviruses in surface water samples was in contrast to data reported previously by other groups (1, 21, 27), who found similar frequencies for adeno- and rotaviruses. This discrepancy might be explained by virus characteristics like resistance to environmental influences and/or fewer circulating viruses in this area. Microbiological water quality was much better in pumps than in wells (A. Uesbeck et al., unpublished data), but viral contamination of two pumps (8%) indicates different levels of water quality. Even though the use of pumps improves the microbiological water quality dramatically, viruses can still be found. We demonstrated that the presence of latrines within a 50-m radius of the testing sites was a significant risk factor for viral contamination of pumps and wells. This distance is in concordance with findings during an outbreak of hepatitis A virus conferred by a lacking sewage tank 60 m apart from water wells. It was even hypothesized that viruses under optimized conditions can travel distances greater than 1,000 m (31). Although the risk of microbiological contamination by latrines in the neighborhood of drinking water sources is recognized in developed countries, it is not broadly recognized in most developing countries (4, 9, 31). In this particular case, the increasing socioeconomic properties in the rural area analyzed led to an increase in the number of latrines, which decreased the risk of human infections by avoiding direct contact with feces. On the other hand, the increase also contributed to contaminating surrounding drinking water sources. These findings demonstrated the importance of observing a balance between medical needs with respect to all kinds of health hazards and local conditions during development.

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